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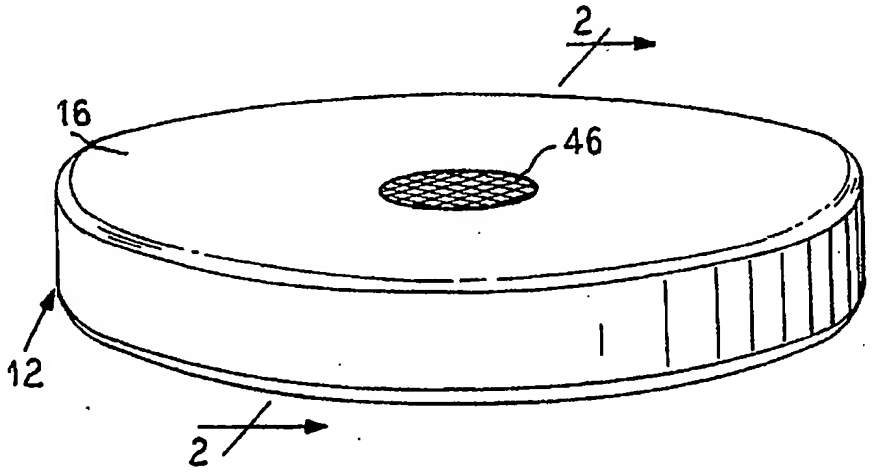

(57) Abstract

An implantable biological fluid measuring device for determining the presence and the amounts of substances in a biological fluid without the need for dilution of the fluid comprises a main housing (12) including electronic circuit means, at least two electrodes and a membrane. An enzyme electrode is also disclosed comprising a multilayered homogeneous monolithic membrane and an intermediate water-swellable coating.

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IMPLANTABLE BIOLOGICAL FLUID MEASURING DEVICE

Cross Reference to Related Application

This application is a continuation-in-part of co-pending application Serial Number 216,683, filed July 7, 1988 and entitled "Biological Fluid Measuring Device" which was a continuation-in-part of pending application Serial Number 852,343, filed April 15, 1986, which was a continuation-in-part of application Serial Number 774,330, filed September 10, 1985 and entitled "Enzyme Electrode."

Technical Field

The present invention relates to implantable devices having membranes which cooperate with an electrode assembly to determine the amount of a substance in a biological fluid.

Background of the Invention

The continuous measurement of substances in biological fluids is of interest in the study and control of metabolic disorders. Electrode systems have been developed for this purpose whereby an enzyme-catalyzed reaction is monitored by an electrochemical sensor. In such electrode systems, the electrochemical sensor comprises an electrode with potentiometric or amperometric function in close contact with a thin layer containing an enzyme in dissolved or insoluble form. The thin layer may also include a co-enzyme.

In conventional practice, a semipermeable membrane separates the thin layer of the electrode containing the enzyme from the sample of biological fluid that includes the substance to be measured. The electrochemical sensor measures the concentration of the substance involved in the enzyme reaction. For example, the concentration of a co-enzyme or a reaction product can be determined. This concentration may be related to the substrate concentration

in the sample by its stoichiometric relationship and by calibration of the electrode system.

A number of enzyme electrodes have been developed, and the operation of those electrodes varies depending on the nature of the enzyme reaction and the particular substance being measured. For example, enzyme electrodes include those that measure: (1) a reactant or product of the enzyme reaction; (2) the consumption of a co-enzyme based on the decrease of its initial concentration and (3) the amount of the reduced or oxidized form of a co-enzyme produced during the enzyme reaction.

The operation of a particular enzyme electrode depends on a number of parameters including diffusion processes, kinetics of the enzyme reaction and the type of electrochemical sensor. In particular, the operation of the electrode can be affected by the diffusion of substances through the semipermeable membrane.

Electrode systems that include enzymes have been used to convert amperometrically inactive substances into reaction products which are amperometrically active. Specifically, in the analysis of blood for glucose content, glucose (which is relatively inactive amperometrically) may be catalytically covered by the enzyme glucose oxidase into the presence of oxygen and water to gluconic acid and hydrogen peroxide. Hydrogen peroxide is anodically active and produces a current which is proportional to the concentration of hydrogen peroxide in the blood sample and thus to the concentration of glucose in the sample.

In a sample of undiluted whole blood, however, a molar excess of plasma glucose is present relative to the amount of plasma oxygen. As a result, if a semipermeable membrane is not included over the enzyme, the concentration of glucose in the sample relative to the concentration of

oxygen will be so high that the glucose oxidase-catalyzed reaction of glucose and oxygen to gluconic acid and hydrogen peroxide will be oxygen limited. The problem can become even greater with implantable devices.

The effect of an oxygen limited reaction is that the range of glucose concentrations that can be measured with such an electrode is very limited. In particular, linearity is not achieved above minimal concentrations of glucose. In a clinical setting, linear glucose levels must be obtained at glucose concentrations of at least up to about 500 milligrams per deciliter (mg/dl). Without a semipermeable membrane over the enzyme, linear glucose levels can be obtained only up to about 40 mg/dl. Thus, the purpose of the membrane over the enzyme in a glucose sensing electrode system is to limit the amount of glucose that passes or diffuses through the membrane. This extends the upper limit of linearity of glucose measurement from a low value without the membrane to a high value with the membrane.

The two fundamental diffusion processes by which a semipermeable membrane can limit the amount of a substance that passes therethrough are diffusion through the semipermeable membrane as a monolithic, homogeneous structure and diffusion through the semipermeable membrane as a porous structure. The processes of diffusion of substances through these different types of membranes differ considerably.

A semipermeable membrane can comprise a porous structure consisting of a relatively impermeable matrix that includes a plurality of "microholes" or pores of molecular dimensions. Transfer through these membranes is primarily due to passage of substances through the pores. In other words, the membrane acts as a microporous barrier or sieve.

Examples of materials that may be used to form such membranes include polyethylene, polyvinylchloride, tetrafluoroethylene, polypropylene, cellophane, polyacrylamide, cellulose acetate, polymethyl methacrylate, silicone polymers, polycarbonate, cuprophane and collagen.

Selectivity in such a membrane can be explained on the basis of the molecular size of the diffusing substances. For substances much smaller than the diameter of the pores, passage of the substance through the membrane is relatively unimpeded. As the effective molecular diameter of the substance approaches the diameter of the pore, the pore will exert a drag on the diffusing substance, reducing its permeability to a value lower than that expected on the basis of the membrane porosity. If the molecules of the substance are too large, they will not pass through the membrane at all.

Since transfer is due primarily to passage of the substance through pores, the permeability is directly related to the size of the pores and to the molecular volume of the diffusing substance. As a result, there is little selectivity in the separation of two chemically or structurally related molecules, except when their molecular size is approximately the same as the size of the pore. When this occurs, there is the possibility that forces acting between the substance and the surface of the pore channel may influence the rate of transfer.

Also, the upper size limit to diffusion will be determined by the largest pore diameter, and the overall diffusion rate will depend on the total number of pores for movement of the substance.

Passage of a substance through a monolithic, homogeneous membrane, on the other hand, depends upon selective dissolution and diffusion of the substance as a

solute through a solid, non-porous film. As used herein, the term "monolithic" means substantially non-porous and having a generally unbroken surface. The term "homogeneous", with reference to a membrane, means having substantially uniform characteristics from one side of the membrane to the other. However, a membrane may have heterogeneous structural domains, for example, created by using block copolymers, and still be characterized functionally as homogeneous with respect to its dependence upon dissolution rather than sieving to effect separation of substances. A monolithic membrane can thus be used to selectively separate components of a solution on the basis of properties other than the size, shape and density of the diffusing substances. The membrane acts as a barrier because of the preferential diffusion therethrough of some substance (a solute).

Despite advances in membrane technology, devices that include semipermeable membranes which have been used to detect and measure the presence of a substance in a biological fluid have generally been restricted to laboratory environments. This is because the devices are generally large and complex and require extensive training to operate. In addition, these devices have been somewhat limited because of the difficulty in replacing a membrane used with the electrode.

A need exists for an improved device that selectively measures the presence and the amounts of particular substances in biological fluids. Such a device should accurately measure the amount of substance in a sample without dilution or pretreatment of the sample. In addition, a basis for selecting appropriate membrane materials for use in such devices is needed. The device should also be implantable within a body to provide

continuous, or intermittent monitoring of a patient. The present invention meets these desires.

Summary of the Invention

The present invention relates to an implantable biological fluid measuring device which permits rapid and accurate determination and measurement of the amount of a particular substance in a biological fluid such as blood or interstitial fluid.

Generally, the device includes a main housing carrying electronic circuit means and at least two electrodes. A membrane is operably associated with the electrodes when the cartridge is mounted on the housing. The membrane includes an enzyme which reacts with a reactant and a co-reactant which is present in a biological fluid. One example is the enzyme glucose oxidase where the reactant is glucose and the co-reactant is oxygen. The membrane has different diffusion rates for the reactant and the co-reactant such that the amount of reactant reaching the enzyme is limited and the amount of co-reactant reaching the enzyme is not depleted. For example, in the case of glucose oxidase, glucose and oxygen is reacted to form gluconic acid and hydrogen peroxide. Particularly in the case of implantable devices, the amount of oxygen which may be present in the biological fluid will be limited. Without any limitation in the amount of glucose reaching the enzyme, the reaction becomes limiting because of the relatively lower amount of oxygen which is present.

To deal with this problem, the membrane relies on differential diffusion, that is, different diffusion rates for the reactant and the co-reactant. The amount of differential diffusion chosen is a function of the biological fluid being tested and its expected ranges for the reactant and the co-reactant. Thus, by limiting the amount of reactant reaching the enzyme by differential

diffusion, the problem of rate limiting or co-reactant limitation is avoided. This provides a wider range of possible measurements for the reactant in the biological fluid.

The electrodes, the supporting structure for the electrodes, and the membrane together form an electrode assembly. The membrane is a multilayered structure including layers formed of materials such as polyethylene, polyvinylchloride, tetrafluorethylene, polypropylene, cellophane, polyacrylamide, polymethyl methacrylate, silicone polymers, polycarbonate, cuprophane, collagen, polyurethanes and block copolymers thereof. The membrane prevents direct contact of the body fluid with the electrodes, but permits selected substances of the fluid to pass through the membrane for electrochemical reaction with the electrodes. To ensure electrochemical reaction, the surface of the membrane layer nearest the electrode is preferably coated with a water-swellable film to maintain electrolyte at the electrode-membrane interface, and thereby improve the sensitivity of the measurement.

In a preferred embodiment, the membrane is a semi-permeable multilayered membrane having at least one layer formed of a nonporous block copolymer having hydrophobic segments and hydrophilic segments that limits the amount of a substance passing therethrough and a second layer including an enzyme that reacts with the substance to form a product.

In a more preferred embodiment, the electrode assembly comprises an electrode, a first (outer) layer of a block copolymer that limits the amount of a hydrophilic substance passing therethrough, a second (intermediate) layer of a block copolymer including an enzyme bound to the first layer and a third (inner) layer of a block copolymer

bound to the second layer and covering the surface of the electrode. The third layer is permeable to relatively low molecular weight substances, such as hydrogen peroxide, but restricts the passage of higher molecular weight substances.

As an alternative, the first (outer) layer itself can be formed of two separate layers to provide the desired selectivity.

In a particularly preferred embodiment, the unbound surface of the third (inner) layer is coated with a semipermeable, substantially solid water-swellable gel-like film. The film comprises the aqueous reaction product of a polyurethane having anionic carboxyl functional groups and non-ionic hydrophilic polyether groups crosslinked in the presence of polyvinylpyrrolidone. The coating, which preferably has a dry film thickness of about 0.1 mil to about 0.5 mil, enhances and maintains the selectivity of the molecular separation of the inner layer and thereby improves the sensitivity of the measured amount of product.

The preferred polymers which form the above-described membrane layers and the coating are selected and based on permeability and water swelling. An accepted industry test procedure for determining the permeability of a coating or membrane is ASTM E 96 which measures the moisture-vapor transmission rate of a material. (American Society for Testing and Materials, Philadelphia, PA).

As used herein, the moisture-vapor transmission rate (MVTR) of a membrane material is expressed in grams per square meter per 24 hours and is one means of defining the water resistance of a material.

The MVTR of a material may be expressed by the equation:

$$MVTR = \frac{Q}{at}$$

wherein the letter "Q" represents the amount of water vapor (in grams) that permeates the film; the letter "a" represents the film area (in square centimeters) and the letter "t" represents the time (in hours at a designated thickness). This value can be converted to grams of water per square meters per 24 hours. The MVTR values identified herein are for membranes that are about 1 mil thick.

The MVTR of the first (outer) layer described herein should be greater than about 4,000 grams per square meter per 24 hours, preferably greater than about 5,000 grams per square meter per 24 hours.

The MVTR of the third (inner) layer of the assembly should be from about 500 to about 4,000 grams per square meter per 24 hours, preferably from 1,000 to about 3,500 grams per square meter per 24 hours.

It will, of course, be understood that the above MVTR values for each layer can be varied or optimized depending on the substance to be measured and the enzyme that is employed.

In a preferred embodiment, the enzyme is glucose oxidase and the substance to be measured is glucose. The amount of glucose in a body fluid is determined by measuring the amount of hydrogen peroxide produced during the oxidation of glucose to gluconic acid by the enzyme.

Preferred polymers for the membrane layers may also be selected by studying water uptake or the swelling of the polymer. This is normally measured by soaking the polymer sample in water at a controlled temperature and exposure conditions until equilibrium is achieved followed by rapid drying of surface water and weighing of the polymer sample. Subtracting the dry weight from the swelled weight and then dividing by the dry weight and multiplying the value obtained by 100 provides the swell rate as a percent

of dry weight. The swell rate of the first (outer) layer described herein should be greater than about 5 percent and preferably greater than about 10 percent. The swell rate of the third (inner) layer should be less than about 5 percent preferably less than about 3 percent.

The swell rate of the coating should be greater than about 5 percent and preferably greater than about 10 percent.

The present invention, however, is not limited to the measurement of glucose concentrations, and other enzyme-substrate systems can be used. Examples of other enzymes include galactose oxidase, uricase, cholesterol oxidase, alcohol oxidase, lactose oxidase, L-amino acid oxidase, D-amino acid oxidase, xanthine oxidase and ascorbic acid oxidase.

Nonetheless, to demonstrate the improvement of this invention over other membrane systems, the invention will be described in terms of measuring glucose concentrations based on the production of hydrogen peroxide by the action of glucose oxidase.

The membrane systems currently available are based on semipermeable membranes with microholes or pores. With these membranes there is little selectivity in the separation of substances that are rather close in size, except when the molecular diameters of the substances approach the diameters of the pores. When this occurs, forces between the substance and the surface of the pore channel may influence the rate of transfer.

The layers of the preferred multilayered membrane described herein each comprise homogeneous, monolithic membranes and differ in composition, structure and operation from conventional microporous membranes. This represents a substantial improvement over current membrane

systems in terms of ease of manufacturing, lifetime of enzyme activity, and the ability to measure the concentrations of substances in undiluted samples.

In addition, the water-swellable coating on the layer of the membrane closest to the electrode represents a substantial improvement in sensor sensitivity by maintaining electrolyte in the electrolyte space at the membrane-electrode interface. This improvement also provides a more stable operation of the device by overcoming electrode start-up problems and drifting problems caused by inadequate electrolyte and the excessive hydrophobicity of the interface environment. Also, by coating the membrane in the above manner, the yield of usable membrane manufactured increases.

Thus, the sensitivity of the device of this invention is improved by the use of a multilayered membrane having the unbound surface of its inner layer coated intermediate to and covering the electrode and by maintaining the membrane in contact with the electrode by osmotic pressure during use. This improvement represents a substantial advantage over current membrane devices in terms of sensor sensitivity, stability of operation, overcoming electrode start-up problems and overcoming interference from mechanical or osmotic disturbances at the electrode-membrane interface.

In summary, passage of substances through the membranes described herein depends upon dissolution and diffusion of the substance through a solid, non-porous film. Components of a solution can be separated on the basis of properties other than the size, shape and density of the diffusing substance.

Brief Description of the Drawings

FIGURE 1 is a perspective view of biological fluid measuring device of the present invention;

FIGURE 2 is a cross-sectional view of the device of FIGURE 1 taken along plane 2-2 of FIGURE 1;

FIGURE 3 is a representation used for modeling purposes;

FIGURE 4 is a graph;

FIGURE 5 is a graph;

FIGURE 6 is a graph;

FIGURE 7 is a graph;

FIGURE 8 is a graph; and

FIGURE 9 is a graph.

Detailed Description of the Invention

The present invention relates to a biological fluid measuring device which permits rapid and accurate measurement of the amount of a particular substance in a biological fluid. One particular use of the present invention is to determine the level of glucose in a body fluid within the body. This is a particularly important measurement for individuals having diabetes, and the device is a substantial development over devices that are now being used by individuals with diabetes to determine glucose levels.

Referring to FIGURES 1 and 2, the measuring device comprises a main housing 12. The housing 12 includes a case 16 having an upper portion 18 and a lower portion 22. The upper portion 18 and lower portion 22 can be connected together by any particular fastening means such as by being hermetically sealed.

The main housing 12 also includes electronic circuit means 24 which can be carried in part on a circuit board (not shown). The electronic circuit means is

preferably maintained in a cavity 26 which is defined by the case 16. The housing also includes at least two electrodes. In the embodiment shown in FIGURE 2, three electrodes 28, 30 and 32 are shown. The operation of these electrodes is discussed in more detail below.

A membrane 34 is operably associated with the electrodes 28, 30 and 32. The electrodes 28, 30 and 32 preferably extend upwardly from a base surface 58. The electrodes are preferably mounted within a post 60 which supports the electrodes as they extend upwardly of the base surface 58. The post is preferably generally circular in design with the interior portion thereof filled with an electrically nonconductive support material such as a hardened polyepoxide-containing resin. The electrically nonconductive support material and the top portions of the electrodes define a membrane contact surface 64. The membrane contact surface 64 is preferably generally dome-shaped such that the membrane 34 can be stretched over the contact surface to more effectively place the membrane in operative association with the electrodes.

In order for the sensitivity of the electrode to function properly, electrolyte must be present and maintained between the membrane 34 and the electrodes at the membrane contact surface 64. In prior devices, variations in electrolyte volume from inconsistent osmotic pressure above the membrane could result in loss of full sensitivity or changes in sensitivity owing to variations in the relatively thin electrolyte layer at the electrode-membrane interface 64. Also, mechanical disturbances could cause changes in the electrolyte media at the electrode-membrane interface 64, where the surface of the membrane and that of the electrode support material (i.e., epoxy resin) were both substantially hydrophobic. In the present device, however,

this problem is overcome by including a water-swellable coating on the surface of the membrane layer nearest to and covering the electrode as discussed in more detail below. Alternatively, this coating can be applied to the electrode-membrane interface 64, but for convenience, the coating is preferably applied to the disposable and more easily renewable membrane.

The water-swellable coating on the surface of the membrane layer at the membrane contact surface 64 provides a substantially consistent electrolyte volume. This improves the sensitivity of the measurement by about 2:1 over that of prior devices. In addition, less sensitivity drift is seen providing a more stable operation. Unlike prior devices using standard membranes, the device of this invention using the coated membrane provides adequate signals to the sensory microcomputer during start-up procedures.

The electrodes 28, 30 and 32 together with a support assembly such as the post 60 and the membrane 34 comprise the electrode assembly. It is this assembly which is contacted with the body fluid for analysis. The electrode assembly 74 is operably associated with the electronic circuit means which analyzes the current from the reaction of the components in the body fluid with the electrodes. The electronic circuit means is in turn operably associated with transmission means such as a radio transmitter to indicate amount of glucose in the body fluid to a receiver (not shown) outside the body.

As shown in FIGURE 2, the membrane 34 is stretched over the post and held in place by a rubber o-ring 42. Additionally provided is a membrane retainer or seal 44 which is mounted on the upper portion 18 of the case 16. To protect the membrane 34 during use or implantation, a screen or fabric 46 is mounted on the upper

portion 18 of the case 16. The screen provides a physical barrier to possible damage to the membrane while still permitting the free flow of body liquids.

The electronics 24 are operably associated with the electrodes 28, 30 and 32 to provide for calibration and readout. The battery is also mounted within the case to power the electronics. The electronics 24 include the necessary circuiting for electrode support, timing and control as well as a transmitter to transmit a signal outside the body to report on the condition of the patient.

In operation, a receiver is placed outside the patient's body to receive the transmitter signals. These can then be interpreted to determine the insulin needs for a diabetic patient and the insulin can then be delivered to the patient. Such a delivery system is discussed in co-ending application Serial Number 513,993, filed April 26, 1990, and entitled "Wearable Blood Glucose Monitor" and is incorporated herein by reference. Alternatively, a supply of insulin can be included in the case 16 and delivered directly to the patient. Also incorporated herein by reference is co-pending application Serial Number 216,683, filed July 7, 1988, entitled "Biological Fluid Measuring Device."

The membrane 34 operates by differential diffusion. It limits the amount of diffusion of the reactant compared to the co-reactant such that a sufficient amount of co-reactant to allow enzymatic reaction with the reactant. In the case of the glucose and oxygen, the amount of glucose passing through the membrane is limited such that a sufficient amount of oxygen is present for the enzyme electrode to operate over a wide range. This solves the problem of relatively low oxygen presence in biological fluids such as found in the interstitial space.

The desired oxygen influx is achieved by placing abundant hydrophobic domains in the structure of the membrane while at the same time limiting glucose influx by limiting hyrodophilic domains. Thus oxygen, which is a hydrophobic gas, readily passes through the membrane while the influx of glucose, a hydrophilic substance, is retarded by the limited number of hydrophilic domains. This provides a glucose enzyme electrode sensor which is no longer oxygen limited even when it is placed in a low oxygen environment such as the interstitium of subcutaneous tissues.

To protect the membrane from any mechanical force which may interrupt the supply of body fluid or the operation of the membrane, it is recessed within the case 16 and protected by the screen 46. Alternatively, the membrane can also be protected by a rigid macroporous cage constructed from biocompatible materials which can absorb any mechanical force. It is also useful if the membrane is oriented such that mechanical force is unlikely to be applied to the membrane.

As shown in FIGURE 2, a dome shape for the post 60 in the membrane 34 is preferred. This is particularly advantageous because it allows the membrane 34 to be stretched tightly over the post and in contact with the electrodes 28, 30 and 32.

It is also possible to treat the screen 46 and membrane 34 with the biologically active material before implantation. One example would be an angiogenesis factor which would cause capillaries to form adjacent to the membrane. Alternatively, an endotoxin can be used to cause capillaries to become relatively leaky or it may be possible to induce bacillary angiomatosis to cause capillary tumor formation adjacent the membrane.

As shown in FIGURE 2, the implant can be circular and relatively flat to facilitate implantation. Alternatively, an oval shape can also be used. Application of a coating or jacket such as surgical dacron fabric will help hold the implant in a stable position and invite the stabilization a well-vasculized tissue capsule in intimate contact with the membrane portion.

The three electrode configuration in combination with the osmotic pressure across the membrane and the chemical reactions occurring in the multilayered membrane, its coating and on the electrode make possible consistent electrode behavior and, in particular, performance of the reference electrode that is stable with time. It is well known in the art that silver/silver chloride electrodes provides a stable reference system for electrochemical sensors.

A silver/silver chloride electrode is typically formed by treating a silver surface with an oxidant and chloride ions (such as by treatment with ferric chloride or a neutral hypochlorite solution), by electrochemical plating of chloride ions onto a silver surface or by the mechanical forming of silver and silver chloride by sintering or similar processes.

When this type of electrode is used in a two electrode configuration with the reference cathode, chloride ions will be lost from the reference electrode which eventually leads to unstable electrode behavior. According to the present invention, permanent stable reference electrode behavior is achieved when the hydrogen peroxide produced in the membrane oxidizes the silver metal to silver oxide which is then converted to silver chloride by chloride ion. Advantages include ease of manufacturing of the electrode, self-forming and self-maintaining

electrode behavior and long-term reference electrode stability.

The relatively low power needs of the present electrode system, as compared to the relatively high power needs of conventional light reflectance-based methods, permit use of a very compact, lightweight device having an extended battery life. CMOS circuitry is used throughout the device and provides a use-dependent battery life of one to two years.

A representative electronic circuit for the device is shown in co-pending application, Serial No. 216,683, filed July 7, 1988, entitled "Biological Fluid Measuring Device," incorporated herein by reference. Other circuits may also be employed. See, for example, Implantable Sensors for Closed Loop Prosthetic Systems, edited by Wen H. Ko, Ch. 12, pages 167-175, Futura Publishing Co., Mount Kisco, N.Y. (1985), the noted relevant pages of which are incorporated herein by reference.

During operation of the device, glucose from the blood sample produces a current flow at the working electrode 28. Equal current is provided by a counter electrode 30 in a reference circuit. The current is converted in an analog section by a current to voltage converter to a voltage which is inverted, level-shifted and delivered to an Analog/Digital (A/D) converter 86 in a microprocessor. As part of the calibration circuit means, the microprocessor can set the analog gain via its control port 90. The A/D converter is activated at one second intervals. The microprocessor looks at the converter output with any number of pattern recognition algorithms known to those skilled in the art until a glucose peak is identified. A timer is then activated for about 30 seconds at the end of which time the difference between the first

and last electrode current values is calculated. This difference is then divided by the value stored in the memory during instrument calibration and is then multiplied by the calibration glucose concentration. The glucose value in milligram percent or millimoles per liter is then transmitted to the receiver outside the patient.

As indicated above the membrane is a multilayered structure including layers formed of materials such as polyethylene, polyvinylchloride, tetrafluoroethylene, polypropylene, cellophane, polyacrylamide, polymethyl methacrylate, silicone polymers, polycarbonate, cuprophane, collagen, polyurethanes and block copolymers thereof.

The layer of the multilayered membrane that is intended to be nearest to and cover the electrode can be coated with a semipermeable water-swellable, substantially solid gel-like film to maintain hydrophilicity at the electrode-membrane interface. This coating also enhances the stability of the third layer of this invention by protecting and supporting the third layer. The electrolyte between a hydrophobic membrane and electrode may experience a large pH gradient due to the electrochemical activity of the electrode, thus damaging the third layer. The buffered electrolyte solution contained in this additional hydrophilic coating adjacent to the third layer protects against such pH-mediated damage. In addition, higher manufacturing yields of usable membranes are achieved by coating the membrane as disclosed herein.

Preferably the coating comprises a flexible water-swellable film having a "dry film" thickness of about 0.1 mil to about 0.5 mil, preferably about 0.25 mil. "Dry film" thickness means the thickness of a cured film cast from a coating formulation onto the surface of the membrane by

coating techniques known in the coating arts. The coating formulation comprises a premix of film-forming polymers and a crosslinking agent and is curable upon the application of moderate heat.

Suitable coatings are formed of a curable copolymer of a urethane polymer and a hydrophilic film-forming polymer. Particularly preferred coatings are formed of a polyurethane polymer having anionic carboxylate functional groups and non-ionic hydrophilic polyether segments, which is crosslinked in the present of polyvinylpyrrolidone and cured at a moderate temperature of about 50 degrees C (about 122 degrees F).

Particularly suitable for this purpose are aqueous dispersions of fully reacted colloidal polyurethane polymers having cross-linkable carboxyl functionality sold under the trademark BAYBOND by Mobay Corporation, a Bayer U.S.A., Inc. Company, Coatings Division (Pittsburgh, PA). These polymers are supplied in dispersion grades having a polycarbonate - polyurethane backbone containing carboxylate groups identified as XW-121 and XW-123; and a polyester-polyurethane backbone containing carboxylate groups, identified as XW-110-2. A detailed description of the properties of these aqueous polyurethane dispersions can be found in the Technical Summary publication Baybond Aqueous Polyurethane Dispersions, published by the Coating Division of Mobay Corporation (undated), the pertinent disclosures of which are incorporated herein by reference.

Particularly preferred is BAYBOND 123, described as an aqueous anionic dispersion of an aliphate polycarbonate urethane polymer and sold as a 35 weight percent solution in water and co-solvent N-methyl-2-pyrrolidone. A description of the properties of BAYBOND 123 is found in the Product Data sheet dated 9/87 and Material

Safety Data Sheet dated 9/7/87 published by the supplier and incorporated herein by reference.

Polyvinylpyrrolidone is also particularly preferred as a hydrophilic water-soluble polymer and is available commercially in a range of viscosity grades and range of number average molecular weights from about 18,000 to about 500,000, under the trade designation PVP K homopolymer series by BASF Wyandotte Corporation (Parsippany, NJ) and by GAF Corporation (New York, NY). Particularly preferred is the homopolymer having a number average molecular weight of about 360,000 identified as PVP-K90 by the suppliers, and sold as a powder.

Also suitable are hydrophilic, film-forming copolymers of N-vinylpyrrolidone, such as a copolymer of N-vinylpyrrolidone and vinyl acetate, a copolymer of N-vinylpyrrolidone, ethylmethacrylate and methacrylic acid monomers, and the like.

The polyurethane polymer is crosslinked in the presence of the polyvinylpyrrolidone by preparing a premix of the polymers and adding a cross-linking agent just prior to the production of the membrane. Suitable cross-linking agents can be carbodiimides, epoxides and melamine/formaldehyde resins. Carbodiimide is preferred. A suitable and preferred carbodiimide crosslinker is sold under the trademark UCARLNK XL-25 by Union Carbide Corporation, Solvent Division (Chicago, IL). The properties of this crosslinking agent are found in the product specification brochure titled "UCARLNK XL-25SE in UCAR PM ACETATE."

The flexibility and hardness of the coating can be varied as desired by varying the dry weight solids of the components in the coating formulation. The term "dry weight solids" means the dry weight percent based on the total

coating composition after the time the crosslinker is included. A preferred useful coating formulation can contain about 6 to about 20 dry weight percent, preferably about 8 dry weight percent, polyvinylpyrrolidone; about 3 to about 10 dry weight percent preferably about 5 dry weight percent cross-linking agent; and about 70 to about 91 weight percent, preferably about 87 weight percent of a polyurethane polymer, preferably a polycarbonate-polyurethane polymer. The reaction product of such a coating formulation is referred to herein as a water-swellable copolymer of polyurethane and polyvinylpyrrolidone.

In a particularly preferred embodiment, the membrane is a semi-permeable multilayered membrane having at least one layer formed of a nonporous block copolymer having hydrophobic segments (such as silicone polymer segments, aromatic and aliphatic polymer segments, polypropylene oxide segments, polytetramethylene oxide segments and the like) and hydrophilic segments (such as polyoxyethylene segments, polyvinylpyrrolidone segments, polyvinyl alcohol segments and the like) that limits the amount of a substance passing therethrough and a second layer including an enzyme that reacts with the substance to form a product.

The first layer limits the amount of a substance in a fluid that can pass therethrough. The substance can react with the enzyme in the second layer to produce one or more reaction products. A third layer that is permeable to one of the reaction products, but which restricts the passage of other materials can also be used.

The ability of each layer to limit the amount of a molecule that can pass therethrough may be expressed in terms of the moisture-vapor transmission rate (MVTR) and water swelling of the material that forms the layer. As

used herein, the MVTR of a material is measured as described in ASTM E 96, the procedure of which is incorporated herein by reference.

The MVTR of the block copolymer of the third layer should be from about 500 to 4,000 grams per square meter per 24 hours. The above values relate specifically to layers that are employed to measure the amount of glucose in a biological sample. It will be understood that block copolymers having different MVTR values can be used to measure the amounts of other substances in a biological sample and the description of glucose measurement is only illustrative.

The most preferred membranes of this invention are formed of polyurethanes which, of course, include urethane groups and the polyurethane ureas which also include urea groups. The polyurethanes and the polyurethane ureas of the present membrane system are based on poly(oxyalkylene) glycols including poly(oxyethylene) glycol. In accordance with conventional usage, both types of polymers will be referred to herein as polyurethanes.

Membranes of polyurethanes based on poly(oxyalkylene) glycol display no predictable relationship between molecular weight and permeability. The unique separation observed with the present membranes may be explained on the basis of substance-membrane or solute-membrane interactions which tend to affect the partitioning is not due only to the hydrophilic poly(oxyalkylene) glycol or "soft" segment, but the hydrophobic or "hard" segment of the block copolymer also contributes to the overall selectivity.

Thus, by changing the structure of the hydrophobic segment of the block copolymer and/or increasing or decreasing the molecular weight of the poly(oxyalkylene)

glycol, the selectivity of the membrane system can be modified. In the membrane system of this invention, for example, the use of two different membranes of block copolyether urethanes based on poly(oxyalkylene) glycol produces the desired selectivity for glucose and hydrogen peroxide.

The preferred poly(oxyalkylene) glycols of this invention include poly(oxyalkylene) glycols, poly(oxytetramethylene) glycols and poly(oxypropylene) glycols. A particularly preferred poly(oxyalkylene) glycol is a poly(oxyethylene) glycol having a weight average molecular weight in the range of about 1,000 to about 4,000.

The organic diisocyanates suitable for use in the preparation of the polyurethanes of the present membranes include 2,4-toluene diisocyanate, 2,6-toluene diisocyanate and 4,4'-diphenylmethane diisocyanate. The use of 4,4'-diphenylmethane diisocyanate is preferred.

Diols useful herein include ethylene glycol, propylene glycol, 1,5-dihydroxypentane, 1,6-dihydroxyhexane, 1,10-dihydroxydecane, 1,4-cyclohexanediol, 1,3-dihydroxyneopentane and alpha, alpha'-dihydroxy-p-xylene.

Diamines useful in the preparation of the polyurethanes described herein include ethylene-diamine, 1,2- (and 1,3-) propanediamine, and methylene-bis-o-chloroaniline.

Set forth below are various examples of membranes which can be constructed to practice the present invention. Examples 1-3 set forth certain base membranes which are combined with the cover membrane set forth in Example 4 to obtain the complete membrane which is used with the present invention. Alternatively, the teachings of Example 4 can be used to modify the membranes of Examples 1-3 such that the

outer layers of the membranes of Examples 1-3 perform the function of the cover membrane set forth in Example 4. This has the advantage of eliminating one layer in the multilayered membrane.

Example 1

The polyurethanes are preferably prepared as block copolymers by solution polymerization techniques as generally described in Lyman, D.J., J. Polymer Sci., 45, 49 (1960). Specifically, a two-step solution polymerization technique is used in which the poly(oxyethylene) glycol is first "capped" by reaction with a diisocyanate to form a macrodiisocyanate. Then the macrodiisocyanate is coupled with a diol (or diamine) and the diisocyanate to form a block copolyetherurethane (or a block copolyurethaneurea). The resulting block copolymers are tough and elastic and may be solution-cast in N,N-dimethylformamide to yield clear films that demonstrate good wet strength when swollen in water.

In particular, a mixture of 8.4 grams (0.006 mole) poly(oxyethylene) glycol (CARBOWAX 1540, Union Carbide Corp., New York, NY) and 3.0 grams (0.012 mole) 4,4'-diphenylmethane diisocyanate in 20 milliliters (ml) dimethyl sulfoxide/4-methyl-2-pentanone (50/50) is placed in a three-necked flask equipped with a stirrer and condenser and protected from moisture. The reaction mixture is stirred and heated at 110 degrees C (230 degrees F) for about one hour. To this clear solution is added 1.5 grams (0.014 mole) 1,5-pentanediol and 2.0 grams (0.008 mole) 4,4'-diphenylmethane diisocyanate.

After heating at 110 degrees C for an additional two hours, the resulting viscous solution is poured into water. The tough, rubbery, white polymer precipitate that forms is chopped in a Waring Blender, washed with water and

dried in a vacuum oven at about 60 degrees C (about 140 degrees F). The yield is essentially quantitative. The inherent viscosity of the copolymer in N,N-dimethyl formamide is 0.59 at 30 degrees C (at a concentration of about 0.05 percent by weight).

Example 2

A membrane formed of a homogeneous, nonporous block copolymer may be prepared as follows. Polymerization is carried out in a 2-liter glass flask with a detachable top containing five inlets. The inlets provide for nitrogen passage, condenser attachment, stirring, thermometer placing, and ingredient addition. A regulated flow of oxygen-free nitrogen passes from a cylinder, through the apparatus, into a water trap, and to the drain. The contents of the reaction flask are stirred by a Teflon blade connected to an electric motor running at 350 rpm. Air is excluded by a mercury seal. Heat is supplied by an electric mantle and temperature recorded by placing a thermometer in the flask contents. A dropping funnel is used for the addition of ingredients during the reaction.

Thirty grams of dimethylaminoethyl methacrylate and 170 grams of acrylonitrile are used. Potassium persulfate is dissolved in 40 milliliters distilled water and portions of the solution are added in sequence with the foregoing monomers as described in Muier et al., J. Biomed. Mater. Res., 5, 415-445 (1971) which is incorporated herein by reference.

The temperature of the mixture in the flask is maintained at 45-50 degrees C (113-122 degrees F) for about 6 hours. The reaction product is an off-white plasticized polymer. The product is washed with water, filtered and dried in a desiccator under vacuum to provide an off-white powder. A typical yield is about 28 grams with a

dimethylaminoethyl methacrylate content (as determined from oxygen content analysis) of about 47 percent and an intrinsic viscosity in dimethylformamide at 25 degrees C (77 degrees F) of 1.13 dl/g.

The polymer is dissolved in DMF to provide a 10 percent solution by weight. The solution is filtered under vacuum through a Porosity G1 sintered glass funnel and is stored in a desiccator over phosphorus pentoxide for at least 16 hours. The polymer solution is poured onto a glass plate and is spread as a film by passing a doctor blade across the plate. Solvent evaporation is achieved by maintaining a temperature of 45-50 degrees C for 8 hours in the region of the plate, while solvent vapor is removed by an extractor fan. The membrane is removed from the glass plate by stripping dry or after being soaked with water.

In the enzyme electrode assembly, the membrane layer nearest the anode (the inner layer) comprises a block copolymer, as described above, which is permeable to hydrogen peroxide but which restricts the passage of higher molecular weight substances. This layer has a preferred thickness of less than about 5 microns, more preferably in the range of about 0.1 to about 5 microns and most preferably in the range of about 0.5 to about 3 microns.

The membrane layer nearest the sample (the outer layer) functions as a diffusion barrier to prevent the passage of high molecular weight substances. This layer, also formed of a block copolymer, when used in an electrode assembly to monitor glucose concentrations in a fluid sample, limits the amount of glucose that passes therethrough. This layer has a preferred thickness of less than about 45 microns, more preferably in the range of about 15 to about 40 microns and most preferably in the range of about 20 to about 35 microns.

The second (intermediate) layer that binds the inner and outer layers together includes glucose oxidase, galactose oxidase, uricase or the like combined with a block copolymer of this invention.

The second layer is applied as a thin uniform layer on either the inner or outer membrane layer and the other membrane layer is brought into contact with the second layer to form a multilayered membrane (also referred to as a laminate). The laminate is then dried to cure the enzyme-containing second layer and to bind the layers together.

Example 3

The unbound surface of the inner membrane layer intended to be closest to the electrode and to cover the electrode of a multilayered monolithic membrane formed according to the procedure of Example 2 can be coated with a water-swellable film. This example illustrates a coating comprising a polyurethane having anionic carboxylate functional groups and hydrophilic polyether groups and polyvinylpyrrolidone (PVP) that can be cross linked by carbodiimide as follows.

A coating preparation is prepared comprising a premix of a colloidal aqueous dispersion of particles of a urethane polymer having a polycarbonate-polyurethane (PC-PU) backbone containing carboxylate groups and the water-soluble hydrophilic polymer, PVP, which is crosslinked by the addition of the cross-linking agent just before production of the coated membrane. Example coating formulations are illustrated in the following table.

	A		B		C	
PREMIX	WEIGHT	DRY WEIGHT PERCENT SOLIDS	WEIGHT	DRY WEIGHT PERCENT SOLIDS	WEIGHT	DRY WEIGHT PERCENT SOLIDS
1. PVP (Note 1)	48	6	64	8	160	20
2. PC-PV (Note 2)	260	91	248	87	200	70
CROSS-LINKING AGENT						
3. Carbodi-imide (Note 3)	6	3	10	5	20	10
Totals	314	100	322	100	380	100

Note 1: Aqueous solution containing 12.5 weight percent PVP prepared from Polyvinylpyrrolidone having a number average molecular weight of about 360,000 sold as a powder under the trademark BASF K-90 by BASF Wyandotte Corporation (Parsippany, NJ).

Note 2: Colloidal dispersion of a polycarbonate -polyurethane (PC-PU) polymer at about 35 weight percent solids in a co-solvent mixture of about 53 weight percent water and about 12 weight percent N-methyl-2-pyrrolidone sold under the trademark BAYBOND 123 (or XW-123) by Mobay Corporation, Coatings Division (Pittsburgh, PA). As supplied, the dispersion has a pH of about 7.5-9.0.

Note 3: Carbodiimide sold under the trademark UCARLNK XL-25SE by Union Carbide Corporation, Solvent Division (Chicago, IL) supplied at about 50 weight percent solids in a solvent solution of propylene glycol monomethylether acetate.

The viscosity and pH of the premix can be controlled and maintained during processing and to prolong the pot life by adding water or adjusting the pH with dilute

ammonia solution or an equivalent base prior to adding the crosslinker.

For production, the coating is applied with a Mayer rod into the unbound surface of a multilayered membrane that constitutes the inner layer described in Example 2. The amount of coating applied should cast a film having a "dry film" thickness of about 0.1 mil to about 0.5 mil, preferably about 0.25 mil. The coating is dried above room temperature preferably at about 50 degrees centigrade.

This coating dries to a substantially solid gel-like film that is water swellable to maintain electrolyte between the membrane covering the electrode and the electrode in the electrode assembly during use.

In certain applications, for ease of application in the electrode assembly, an appropriate carrier or frame made of cardboard, rubber or plastic can be secured to the surface of the laminate or multilayered membrane. The frame includes an opening, for example, in the central portion thereof whereby the outer layer of the membrane may be exposed to the electrode.

The electrode assembly of this invention may also be used in the manner commonly employed in the making of amperometric measurements. A sample of the fluid being analyzed is placed in contact with a reference electrode, e.g., silver/silver-chloride, and the electrode of this invention which is preferably formed of platinum. The electrodes are connected to a galvanometer or polarographic instrument and the current is read or recorded upon application of the desired voltage between the electrodes.

Example 4

A membrane suitable for use in the present implantable device comprises the foregoing four layer membrane of Example 3 in contact with the amperometric

electrode. A second membrane which functions as a fifth layer is applied over the four layer membrane during assembly in a conventional manner. The second membrane preferably has a thickness of about 1.2 mil and can comprise a blend of two substantially similar polyurethane urea copolymers, one having a glucose permeability that is somewhat higher than preferred (polymer A) and the other having a glucose permeability that is somewhat lower than preferred (polymer B). The two copolymers comprise polyurethane ureas which include methylene bis(p-phenyl-isocyanate) (MDI) hard segments and ethylene diamine chain extended portions. The respective prepolymers are prepared by solution polymerization in a conventional manner with 80 percent polytetramethylene oxide (PTMEG)/20 percent polyethylene glycol (PEG) for the high permeability soft segment and 85 percent PTMEG/15 percent PEG for the low permeability soft segment. The synthesis is completed by chain extension with ethylene diamine. Blends of the two copolymers A and B are prepared in the range of 1:4 to 2:1 (high:low permeability polymer). The preferred ratio is 1:1.2.

In an alternative embodiment, the foregoing fifth layer can be eliminated and the outermost layer of the four layer membrane described in Example 3 can be modified to provide a membrane which exhibits the desired differential diffusion characteristics. In particular, the thickness of the outermost (relative to the electrode) layer, i.e., the resistive layer, can be increased either by lamination or as originally solvent cast. In this alternative embodiment, a preferred thickness of the outermost layer is less than about 100 microns, more preferably in the range of about 30 to about 80 microns and most preferably in the range of about 40 to about 70 microns. Conventional procedures for

the preparation of polyurethane ureas are described in Lelah et al., Polyurethanes in Medicine, CRC Press, Inc. (1986), the disclosures of which are incorporated herein by reference. Commercial sources of PTMEG, MDI and PEG are well known to those skilled in polymerization arts and are listed in generally available supplier catalogs.

The desired differential diffusion can be determined by study of the materials used to make the membrane. To define the required membrane permeabilities a model is used based on the biological fluid being tested and the reactant and co-reactant. For ease of description the example of glucose and oxygen is given.

The membrane permeability model used the digital simulation approach of Brumleve et al., J. Electroanal. Chem., 90 (1978) 1-31, with modifications made to account for enzyme kinetics. The first step was to subdivide the various membrane compartments and contacting solution regions into finite elements.

Fick's laws of diffusion is then solved to account for species flux after a concentration step. Glucose reaction at the site of immobilized glucose oxidase in the presence of oxygen co-substrate can be described by a variety of kinetic schemes. However, to obtain the widest range of glucose linearity, the enzyme layer was maximally loaded to ascertain that the rate controlling step (when sufficient oxygen is present) was glucose diffusion through a resistance layer into the enzyme layer. This allows the assumption of infinitely fast enzyme kinetics. The basic elemental distance grid is shown in FIGURE 3 for three regions: the contacting solution 1, the outer resistance layer 2, and the enzyme layer 3. The enzyme layer 3 to the left is in contact with the electrode 4 is the solution element 1 is furthest from the electrode.

For a membrane to maintain long-term linearity and stability, oxygen flux from the contacting solution should be able to match glucose flux at concentrations of up to 500 mg/dl glucose (.0275M) when oxygen concentration is as low as 25 mmHg (.00004M). To make up for this large concentration mismatch, a polymer matrix should minimally extract glucose while allowing enhanced extraction of oxygen. An additional advantage can also be gained by a polymer which allows faster oxygen diffusion than glucose diffusion.

In multilayer enzyme sensors, the species diffusion coefficients for the outermost membrane layer (called the resistance layer) should be at least a factor of 50 smaller than the solution diffusion coefficients. This minimizes the potential for species depletion into the solution layer and limits convention artifacts because it maintains constant species concentration at the membrane/solution interface. Literature values for aqueous diffusion coefficients (in cm^2/sec) are $2.1X10^{-3}$ for oxygen, $1.0X10^{-5}$ for hydrogen peroxide, and $3.0x10^{-6}$ for glucose. See Mell et al., Anal. Chem., 47 (1975) 299-307 and Gough et al., J. Electrochem So., 127 (1980) 1278-1286. Thus, maximum membrane diffusion coefficients should be scaled accordingly.

For more extractable species (i.e., K>1) like oxygen, an even larger reduction in diffusion coefficient might be required to avoid solution depletion. As a first approximation, it is assumed that the membrane/solution oxygen surface concentration will not be perturbed. After solving this initial case where the membrane/solution interface remains constant, complications involving surface layers (i.e., stagnant films, clots, tissue interfacial layers, etc.) can be further modeled by adding additional

volume elements with slower diffusion and/or lesser extraction properties and resultant concentration depletion effects.

First the initial approximation is made for the chosen factors. Species flux is defined as $f_i = D_i K_i C_i / L$ where D_i is the species diffusion coefficient, K_i is the species extraction coefficient, C_i is the species concentration in solution at the membrane surface, and L is the membrane thickness. Assuming a 10% safety margin for oxygen to glucose flux gives the following:

$$1.1 \times f_{O2} > f_g$$

$$(1.1 \times D_{O2} \times K_{O2} \times .00004)/L > (D_g \times K_g \times .0275)/L$$

$$(D_{O2} \times K_{O2})/(D_g \times K_g) > (.0275)/(1.1 \times .00004) > 625$$

As can be seen, this calculation is thickness independent. If the polymer used has permeabilities correctly matched, theory indicates that the resistance layer can be made as thin as practicality allows, thus speeding up time response and increasing signal.

As a reference point, this calculation would give the following expression using the parameters calculated from typical permeability measurements on the membrane set forth in Example 3.

$$(6.4 \times 10^{-7} \times 2.5)/(1.6 \times 10^{-7} \times .11) = 90$$

Thus, a reduction on the order of at least 7 will be required in the permeability coefficient of the resistance layer for continuous implantable glucose monitoring under the solution conditions envisioned.

To make permeability measurements, membranes were set up in the configuration of Example 3 and allowed to hydrate for at least two hours. Concentration steps were then performed for glucose, hydrogen peroxide, or dissolved oxygen and the resultant output current transients acquired

on a Mac Plus computer with a Remote Measurement System analog to digital converter. These output transients were then first analyzed by standard methods. See Mancy et al., J. Electroanal. Chem., 4 (1965) 65-92 and then further refined by digital simulation to define the diffusion coefficients and extraction coefficients for each polymer material. The membrane permeability is then simply the product of these two coefficients.

Blending experiments were performed at five evenly spaced compositional variations. The resultant permeability values define a smooth curve. Thus, as shown in the table below, it was possible to reproducibly vary glucose permeability over two orders of magnitude which comfortably bracketed the permeability deemed necessary for the implantable sensor (P_{O2}/P_g ratio > 625). Hydrogen peroxide and oxygen permeability were fairly independent of the polymer blend composition.

Membrane	15.0(1)	"15.9"	"16.5"	"17.3"	"18.1"	"18.9"	20.0(2)
Ratio A/B	0.00	0.22	0.43	0.85	1.63	3.54	∞
P_g X 10^8	<0.01	0.12	0.19	0.45	0.50	0.60	0.81
P_{O2}/P_g	16000	1333	842	355	320	267	197

(1) Membrane is pure B.

(2) Membrane is pure A.

Taking the permeability information described above, one can predict electrode output for a specific set of boundary conditions. For purposes of analysis one can assume that there is a stagnant film .01" thick next to the membrane for which diffusion is the controlling transport mechanism, but any solution beyond this point is of infinite volume and convection rate and therefore its concentration cannot be perturbed. The resultant currents for 250 mg/dl

and 500 mg/dl glucose contacting solutions over 15 minutes as a function of membrane are shown in FIGURE 4. As permeability decreases, the absolute currents go down (FIGURE 5) but linearity is finally established as shown in FIGURE 6 for the "15.9" membrane.

The three electrode configurations prepared for study include:

1. Polymer dip-coated membrane over 0.020" diameter platinum wire (cylindrical),

2. Multi-layered roll-coated polymer membrane press fit over a membrane much in accordance with Example 3, electrode bodies (planar),

3. Multi-layered roll-coated polymer membrane laminated and/or press fit over substrates with vacuum evaporated metallizations (planar).

In the first configuration, platinum wire was epoxy-sealed into glass and the wire then sequentially coated with the polymer layers. This coating included two coats of an interference blocking layer (<0.00005" thick) directly over the platinum, followed by two coats of a <0.0002" thick polymer/enzyme layer, and capped with a terminal layer of two or four coats of a blended resistance layer (<0.0005" thick). Probes were dried at 60°C for 30 minutes between layer applications. These dip-coated probes were then paired with a Pt wire auxiliary electrode and a Ag/AgCl reference electrode and assembled for performance testing.

In the next case, the three electrode assembly was made up as a unit with the Pt and Ag wires being sealed into epoxy and then the epoxy sealant was machine finished to allow the wires to be polished flush to the surface. Instead of sequentially adding the membranes over this assembly, the membranes were formed separately from the

electrodes with each layer being cast and dried to well defined thicknesses on a continuously moving roll. First the resistance layer (0.0005" to 0.0015" thick) was coated over an inert polymer support, followed by the enzyme layer (0.0002" to 0.0004" thick), and then the interference layer (<0.00005"). The resultant composite film was then die cut, and press fit over the electrode assembly for testing.

In the final case, the three electrode assembly is formed by the vacuum sputtering of the appropriate metals onto an inert substrate. Contacts are then made to the leads and all but the center of the electrode metallization encapsulated. The composite membrane in roll form as described above was die cut to the desired electrode shape, adhered to the substrate and then assembled for testing.

In room temperature testing of dip-coated probes, there was a break-in time of approximately two days to come up to a stable glucose response. With polymer ratios (A/B) of 1.63 or less, these prototype electrodes showed usable response from 0 mg/dl to 400 mg/dl glucose approximately 90% of the time. Time responses of less than 45 seconds were typical for concentration step changes. As oxygen tension was lowered to 35 mmHg in the testing, all polymers with an A/B ratio of 0.43 or less showed reductions in signal of 20% or less. However, as oxygen tension was further lowered to 25 mmHg, only the polymer ratio of 0.22 could hold this performance for times over 20 minutes.

The results for the multi-layer roll-coated membranes press-fit over the membrane of Example 3 were best and this is the preferred configuration. In aqueous testing, linearity was good to 400 mg/dl glucose as shown, in FIGURE 7.

Long term stability of better than 2% over 24 hours was typical for 100, 200 and 400 mg/dl glucose.

Temperature sensitivity of approximately 4%/°C was observed and then corrected for in all subsequent measurements. Time response to concentration steps was on the order of three minutes.

For the polymer with an A/B ratio of 0.43, there was no loss of signal in a 400 mg/dl glucose solution until oxygen tension was reduced to below 35 mmHg. When the ratio was further dropped to 0.22, then oxygen tension could be reduced to below 20 mmHg before noticeable loss of signal occurred. This is demonstrated in FIGURE 8.

These electrodes showed highly desirable interference rejection performance with a 100 mg/dl ascorbic acid bolus (20 times blood concentration) resulting in a signal increment of less than 10 mg/dl glucose. In addition, testing of zero glucose blood plasma for several days showed no increase in signal baseline or deactivation of enzyme activity. Finally, there was no significant stirring artifact as signal changed less than 5% in going from unstirred to highly stirred solutions.

Once a week calibrations from 0 to 400 mg/dl glucose showed less than 5% variation over the course of the electrode lifetimes. Electrodes stored in zero glucose solution between measurements showed no sign of loss of electrode calibration over two months. When an accelerated lifetime test was performed on the 0.43 ratio membrane electrodes by storing the electrodes in a 400 mg/dl glucose solution between tests, the electrodes began to lose sensitivity and became increasingly nonlinear at approximately four weeks. Assuming that the average glucose concentration in vivo is 100 mg/dl and that the 0.22 ratio membranes would reduce glucose permeability another 50%, a minimum electrode lifetime of eight months can be projected.

Candidate implantable sensor cover membranes were inserted into the peritoneal cavity of rats and harvested at regular intervals over one year. These were then washed and placed on the membrane of Example 3 for testing. Specification was maintained on all membranes tested. Neither the extended in vivo exposure to glucose nor the suspected reduction of oxygen cofactor cause the enzyme activity to deteriorate.

A generally planar configuration for the membrane is preferred since it is more reproducible, maintains better rejection of interferences and provides low oxygen tension tolerance. In addition, it is linear and stable, with a projected lifetime of at least a year, as verified by in vivo testing.

In vivo testing was conducted for both of the glucose sensor evaluations in dogs. The glucose sensor used a multilayer roll-coated membrane. The immobilized glucose oxidase activity contained in this multilayer membrane survived 370 days of implantation in the peritoneal cavity of the rat as discussed above. A multilayer sensor membrane was constructed as discussed above to produce a glucose sensor.

This sensor was coupled with electronic circuitry to a commercially available (Data Sciences, Minneapolis, Minnesota) implantable radio transmitter and appropriately sealed by a thermally bonded polyethylene housing. These implantation units are disc shaped (6.4 cm in diameter and 0.7 cm in depth), and press fitted into a "tissue ingrowth jacket" made of double velour Dacron^{TM} (polyester) surgical fabric (Meadox, Inc. Oakland, NJ). Each implantation device was tested and showed linearity for glucose from 0-250 mg/dl using standards made up in both phosphate buffer and plasma. Each device was then sterilized in 70% isopropyl alcohol and

surgically implanted in the subcutaneous tissue typically just caudad to the scapula of the dog.

Typically, this implanted sensor has responded appropriately and continuously to bolus intravenous infusion of glucose and insulin and to intramuscular glucagon for 12 weeks. A typical response is shown in FIGURE 9 below, along with the serial simultaneously obtained plasma glucose determinations run using the hexokinase plasma glucose reference method.

By repeating these dynamic glucose studies weekly, it has been shown that the sensor is indeed tracking the glucose concentration of subcutaneous tissue. Analysis of the data shows that the subcutaneous sensor appears to lag the blood glucose response by about eight minutes. The data shown in FIGURE 9 was obtained at the end of the 11th week of implantation and is similar to the response seen during the first week of implantation and on the benchtop prior to implantation. These dogs were not made diabetic and thus, in between glucose tolerance tests the radio transmitters report tissue glucose levels in the normal range.

Several techniques can be used to construct the sensor. The membranes were press fitted over the dome-shaped electrode and be anchored in place using a Teflon O-ring and stainless steel ring clip. A electrolyte solution as discussed above can be placed between the membrane and the electrode.

A porous barrier fabric or screen can be placed at the tip of the sensor. To this fabric the sensor membrane, or both, can be absorbed a highly purified commercially-available extracellular matrix material. See Kleinman et al., Biochemistry, 25 (1986) 312 and the Basement Membrane MATRIGEL™ specification sheet from Collaborative Research, Inc., Bedford, Mass. In turn, to

this material can be absorbed an angiogenesis peptide factor.

To initiate angiogenesis this peptide factor should remain active and at the tip of the sensor for at least 72 hours to be effective. While hypothesis that angiogenesis factor can be used to enhance sensor performance and lifetime needs further study and validation, it appears that it is better to invite angiogenesis rather than fibrocyte proliferation and dense scar tissue.

The ability of the present device assembly to accurately measure the concentration of substances such as glucose over a broad range of concentrations in fluids including undiluted whole blood samples enables the rapid and accurate determination of the concentration of those substances. That information can be employed in the study and control of metabolic disorders including diabetes.

The foregoing is intended as illustrative of the present invention but is not limiting. It should be understood that numerous variations and modifications can be made without departing from the spirit and scope of the novel concepts of the invention.

WHAT IS CLAIMED IS:

1. An implantable biological fluid measuring device comprising:

a) a main housing including electronic circuit means associated with at least two electrodes;

b) a membrane including an enzyme which reacts with a reactant and a co-reactant present in the biological fluid mounted on the housing with the membrane operably associated with the electrodes, the membrane having different diffusion rates for the reactant and the co-reactant such that the amount of reactant reaching the enzyme is limited and the amount of co-reactant reaching the enzyme is not depleted.

2. The biological fluid measuring device of claim 1 wherein the reactant in glucose and the co-reactant is oxygen.

3. The biological fluid measuring device of claim 1 wherein the membrane includes a first layer of nonporous block copolymer having hydrophobic segments and hydrophilic segments that limits the amount of the reactant passing therethrough, a second layer of a nonporous block copolymer including an enzyme bound to the first layer, said enzyme reacting with the substance to form a product, and a third layer of a nonporous block copolymer having hydrophobic and hydrophilic segments that is bound to the second layer, the third layer having a water-swellable coating on the unbound surface intermediate to the electrode and covering the surface of the electrode, so that the third layer restricts the passage of the substance therethrough but permits the passage of the product and the intermediate coating maintains electrolyte at the membrane-electrode interface whereby the measurable amount of product formed corresponds to the amount of the substance in the sample.

4. The biological fluid measuring device of claim 1 further including transmission means carried by the main housing and operably associated with the electronic circuit means for transmitting a result.

5. An enzyme electrode for determining the amount of a reactant in a sample by measuring the amount of a product formed comprising an electrode covered with a multilayered membrane comprising a first layer of a block copolymer that limits the amount of the reactant passing therethrough compared to the amount of a co-reactant passing therethrough, a second layer of a block copolymer including an enzyme bound to the first layer, said enzyme reacting with the substance to form a product, a third layer of a block copolymer bound to the second layer, and having a water-swellable coating intermediate the unbound surface of the third layer and the electrode and covering the surface of the electrodes so that the third layer restricts the passage of the substance therethrough but permits the passage of the product and the intermediate coating maintains electrolyte at the electrode-membrane interface during use, whereby the measurable amount of product formed corresponds to the amount of the substance in the sample.

FIG. 1

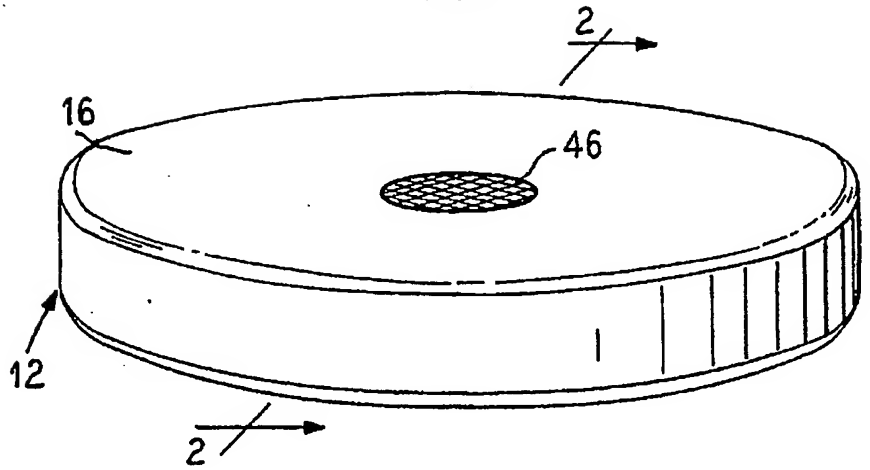


FIG. 2

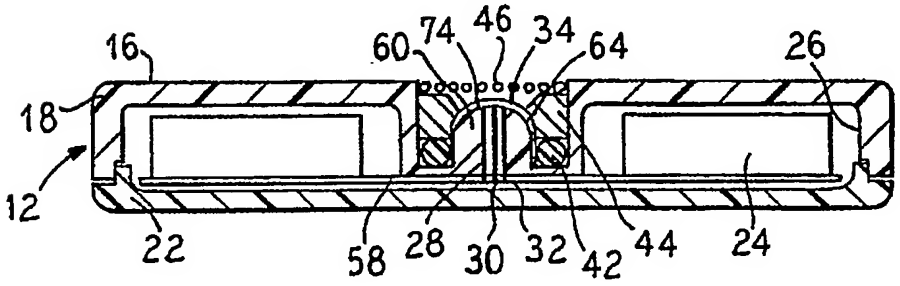


FIG. 3

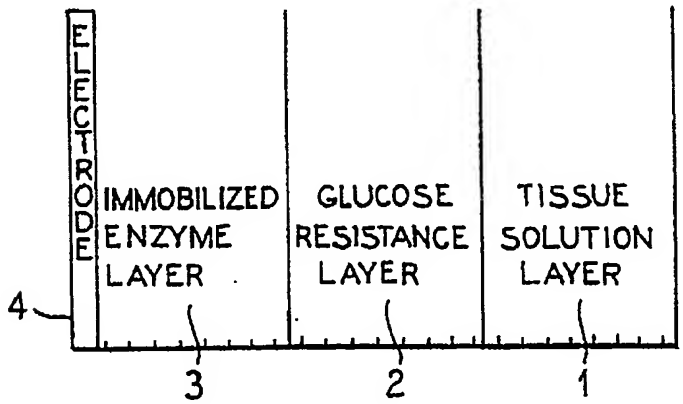


FIG. 4

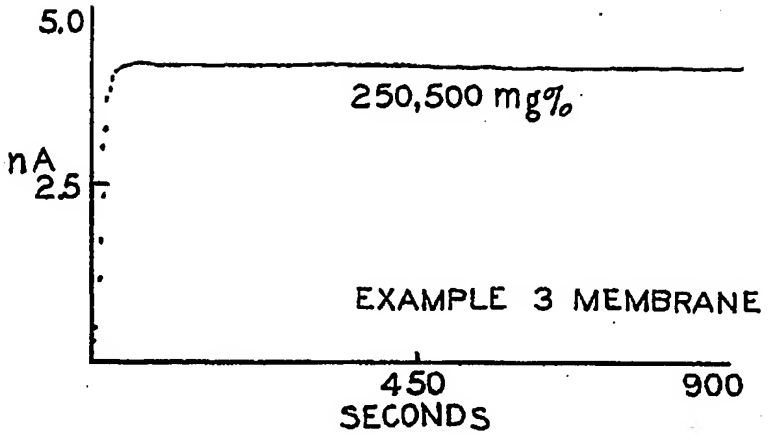


FIG. 5

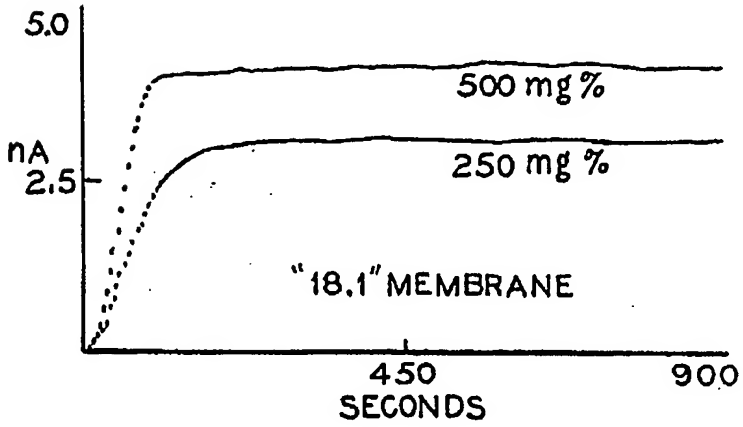


FIG. 6

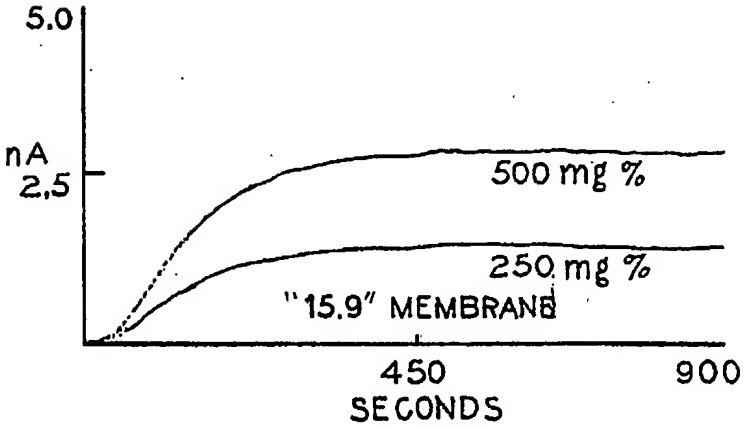


FIG. 7

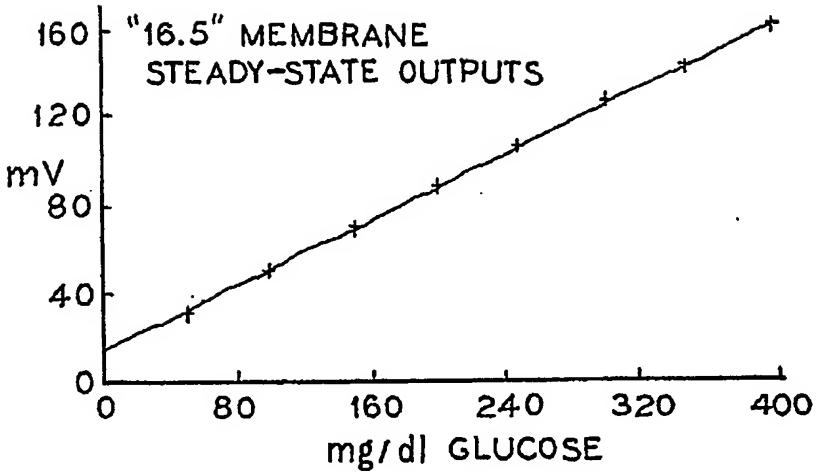


FIG. 8

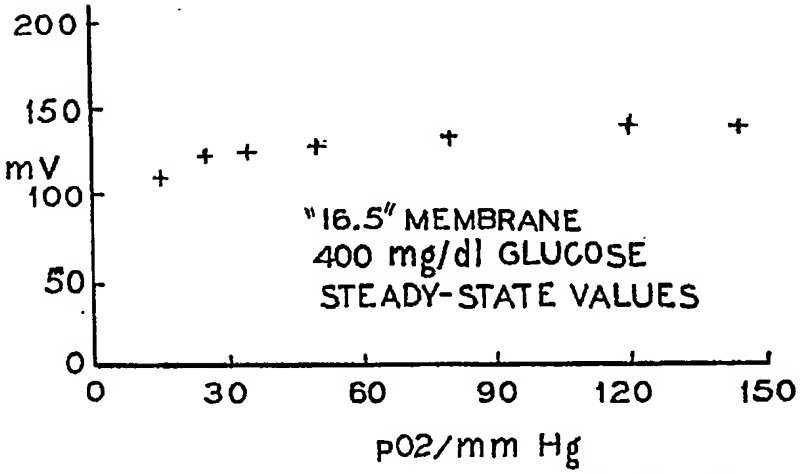
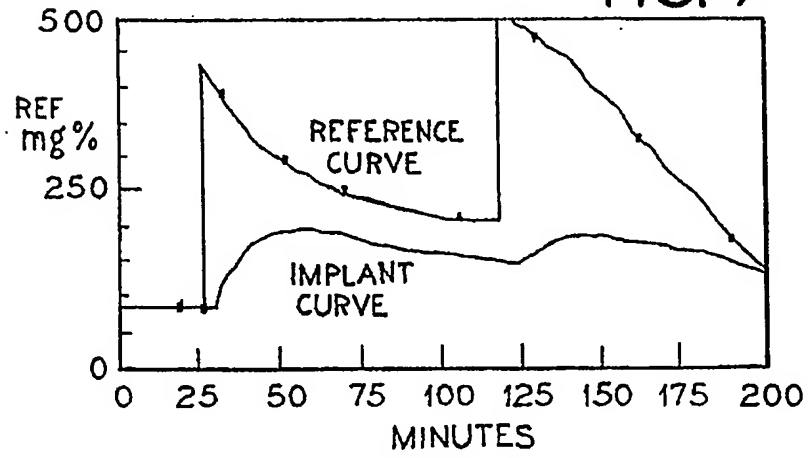


FIG. 9



INTERNATIONAL SEARCH REPORT

International Application No PCT/US92/00626

I. CLASSIFICATION OF SUBJECT MATTER

IPC (5): G01N 27/416
U.S.CL.: 204/403

II. FIELDS SEARCHED

Classification System	Classification Symbols
U.S.	204/403 128/635; 437/817

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	US, A, 4,404,066 (JOHNSON) 13 September 1983 See figures 3(a) and 3(b).	1, 2 and 4
X	US, A, 4,418,148 (OBERHARDT) 29 November 1983 See figures 2-4.	1, 2 and 4
X	GB, A, 1,442,303 (CHRISTIANSEN) 14 July 1973 See entire document.	1, 2 and 4
Y	US, A, 4,280,505 (DALI ET AL.) 28 July 1981 See figure 7 and column 4 lines 24-34.	3 and 5
Y	US, A, 4,317,879 (BUSBY ET AL.) 02 March 1983 See figure 1 and column 4 lines 6-8.	3 and 5
Y	US, A, 4,324,256 (VESTERAGER) 13 April 1982 See figure 2 and column 5 lines 57-62.	3 and 5

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 March 1992	01 JUL 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	N. Nguyen

Form PCT/ISA/210 (second sheet) (Rev.11-87)